REPLICATION PROTEIN A BINDING TRANSCRIPTIONAL FACTOR (RBT1) AND USES THEREOF

BACKGROUND OF THE INVENTION

(a) Field of the Invention

The invention relates to Replication Protein A (RPA) and more particularly to a RPA transcriptional factor to treat neoplastic disorders such as cancer.

(b) Description of Prior Art

Replication Protein A (RPA), also known as replication factor A (RFA), is a ubiquitous and abundant heterotrimeric protein required for DNA replication, repair and recombination in eukaryotes. RPA nonspecifically binds single-stranded DNA and plays an essential role in the regulation of DNA metabolism *via* multiple protein interactions and/or RPA phosphorylation. More particularly, RPA binds single-stranded DNA with strong affinity (association constant of 10^{9}-10^{11} M^{-1})and greatest affinity for polypyrimidine tracts. RPA also binds double-stranded DNA with lower affinity and is likely to facilitate DNA unwinding. RPA may play a role in the regulation of transcription by binding regulatory elements in promoters; in yeast, RPA binds specific regulatory sequences in the promoters of DNA repair and metabolism genes (Singh K. *et al.*, 1995, *Proceedings of the National Academy of Science USA* **92**(11):4907-11).

RPA is made of three subunits: a 70-kDa subunit (RPA70), a 32-kDa middle subunit (RPA32) and a 14-kDa subunit (RPA14). The RPA32 subunit is phosphorylated in a cell cycle-dependent manner.

RPA-protein interactions appear to be largely mediated by the large 70-kDa subunit (RPA70). RPA70 interacts with the p53, GAL4, VP16, EBNA1 and SV40T antigens and with DNA polymerase alpha (Wold, M., 1997,

Annual Review of Biochemistry, "Replication Protein A: A Heterotrimeric, Single-Stranded DNA-binding Protein Required for Eukaryotic DNA Metabolism"). It is also important in interaction with DNA repair proteins involved in damage recognition and excision.

Interaction with XPF stimulates its 5' junction-specific endonuclease activity, interaction with XPG targets this endonuclease to damaged DNA, and interaction with ERCC1 (ERCC1 also binds *xeroderma pigmentosum* group A factor, XPA, which is another NER factor) promotes exonuclease activity.

The possibility of interaction by the aforementioned repair proteins with RPA32 has not been clearly elucidated. However, interactions with some proteins involved in DNA repair appear to be mediated by RPA32, such as interaction with XPA and uracil-DNA glycosylase. A region of significant homology between uracil-DNA glycosylase and XPA was also reported, suggestive of the possibility of a common binding motif to RPA32 across several different proteins. Furthermore, some important protein interactions, such as with RAD52, appear to involve all three subunits of RPA (Hays, S. *et al.*, 1998, *Molecular and Cellular Biology* **18**(7):4400-4406).

In cells, RPA is phosphorylated by DNA-dependent protein kinase (DNA-PK) when RPA is bound to single-strand DNA, during the S phase and after DNA damage; and also possibly by ATM.

Phosphorylation of RPA is observed in a cell-cycle dependent manner and in response to DNA damage (i.e. UV light, X-rays, cisplatin) in eukaryotic systems. This phosphorylation takes place predominantly on the N-terminal region of RPA32 and was previously thought to be effected by DNA-dependent protein kinase (DNA-PK). However, RPA hyperphosphorylation still takes

place in SCID cells where DNA-PK is believed to be responsible for its repair and recombination defects. Ataxia telangiectasia mutated gene (ATM), an important cell cycle checkpoint protein kinase belonging to the same kinase family as DNA-PK, may be responsible for the *in vivo* phosphorylation of RPA32. In *Saccharomyces cerevisiae*, the ATM homolog, MEC1, is essential for RPA phosphorylation. Furthermore, ionizing radiation-induced phosphorylation of RPA32 is deficient and reduced in primary fibroblasts from patients suffering from ataxia telangiectasia in comparison to normal, aged fibroblasts.

The result of RPA32 phosphorylation on DNA metabolism is largely unsolved. It has been noted that IR-induced RPA phosphorylation can be uncoupled from the S-phase checkpoint in ataxia telangiectasia cells, suggesting that RPA phosphorylation in itself is not necessary or sufficient for an S-phase arrest. Phosphorylation, however, may affect the conformation of RPA, thereby modulating its affinity for DNA and its protein interactors, and altering the balance between DNA replication and repair. Hyperphosphorylation of RPA32 *in vivo* is concordant with a decrease in the binding of RPA to single-stranded DNA. This observation is interesting to note since phosphorylated RPA32 is found predominantly in the S-phase of the cell cycle.

RPA has been found to have a high affinity for UV-damaged and cisplatin-damaged DNA and the accompanying phosphorylated form of RPA is correlated strongly with a reduction of the *in vitro* DNA replication activity of the concerned cell extracts.

It would therefore be highly desirable to identify physiologically relevant protein interactors of the RPA32 subunit of Replication Protein A. Identification of such protein interactors would

contribute to the understanding of DNA repair, transcription, and cell signaling. The proteins involved in nucleotide excision repair (NER), for example, are quite numerous and the basis for their interaction and function is not yet completely understood. Understanding the regulation of these pathways would assuredly lend insight into their role in cancer susceptibility. RPA, as a protein involved integrally in modulating DNA repair, replication and recombination, would be key to understanding the connection between and within pathways. The implications to cancer therapeutics and/or prevention would be significant.

SUMMARY OF THE INVENTION

One aim of the present invention is to provide a protein interactor of the RPA32 subunit of Replication Protein A (RPA).

Another aim of the present invention is to provide a RPA transcriptional factor to treat neoplastic disorders such as cancer.

In accordance with the present invention, there is provided a gene having the characteristics of a gene encoded by a nucleotide sequence as set forth in Fig. 1 (SEQ ID NO:1).

The gene may be from a human, a mouse, a rat or a yeast.

In accordance with the present invention, there is also provided a protein having the identifying characteristics of a protein encoded by a nucleotide sequence as set forth in Fig. 1(SEQ ID NO:1).

The protein may be from a human, a mouse, a rat or a yeast.

Antibodies may be raised against the gene.

The gene, replication protein A binding transcriptional activator 1 (RBT1), encodes a protein interactor of the Replication Protein A (RPA). More particularly, a protein interactor of the Replication Protein A 32KD subunit was identified. RBT1 binds RPA32.

The RBT1 gene has a high activity in cancer cells compared to normal cells, may be involved in carcinogenesis and is highly transactivated in cancer cells.

The RBT1 nucleotide and/or amino acid sequences may be used to generate reagents, such as plasmids, antibodies and inhibitors, including antisense/ antibodies which may be used in treating neoplastic disorders such as cancer.

The RBT1 sequence of the present invention may also be used for the preparation of a medicament for gene therapy, wherein he RBT1 sequence is used as a specific promoter to overexpress genes of interest in specific tissues.

In accordance with another embodiment of the present invention, there is provided a method of gene therapy, which comprises the use of RBT1 sequence as a promoter for overexpressing a gene in a suitable tissue.

The RBT1 gene may further be used to induce apoptosis in cells such as cancerous cells, by modulating its expression using molecular or chemical approaches.

The RBT1 sequence of the present invention may also be used to develop antisenses and/or inhibitors to treat diseases including cancers and leukemia.

BRIEF DESCRIPTION OF THE DRAWING

Fig. 1 illustrates the nucleotide (SEQ ID NO:1) and the amino acid sequence (SEQ ID NO:2) of RBT1.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, there is provided a gene sequence encoding a protein interactor of Replication Protein A, identified using the yeast two-hybrid system. The gene, named RPA Binding Transcriptional Activator 1 (RBT1), has a putative open reading frame of 196 amino acids. The coding sequence of RBT1 corresponds to several expressed sequence tags (ESTs), including one derived from an ovary tumor cell line. The gene of the present invention acts as a strong transcriptional activator in yeast and mammalian cells. Furthermore, transcriptional activation, as assayed by a luciferase reporter gene, demonstrated that the activity of the RBT1 gene of the present invention is higher in cancer cells compared to normal non-immortalized cells. RBT1 expression is higher in cancer cells compared to normal cells. More particularly, a protein interactor of Human Replication Protein A 32 (RPA32) was identified.

BLASTP homology searches against the deduced amino acid sequence of RBT1 reveal that it is an undefined protein with little homology to known protein sequences. Further, BLASTN homology searches only identified approximately 20 human expressed sequence tags (ESTs) which had high homology to RBT1.

Northern blot using an RBT1 DNA probe showed one transcript of approximately 1.55 kb in size. *In silicio* analysis suggested that RBT1 consists of an open reading frame (ORF) of 196 amino acids and a theorectical molecular weight of 22 kDa. This is in agreement with Western Blot analysis.

Differential expression of RBT1 was also investigated as it relates to cancer. Semi-quantitative analysis has shown that RBT1 is at least ten times more expressed in cell line H661 (cancer cells) than NHBEC (normal cells).

Various cell lines are investigated to ascertain whether RBT1 has relevance to carcinogenesis. *In silicio* analysis also suggests that the N-terminal domain of RBT1 contains a putative DNA binding domain. Whether RBT1 binds specific DNA regulatory elements is also being investigated.

The presence of an acidic domain in the C-terminal domain of RBT1 led to investigate whether RBT1 was a potential transcriptional activator. RBT1, fused to the LexA binding domain, strongly promotes transcription of reporter genes LacZ and HIS3 in the yeast two-hybrid system, suggesting its possible role as a transcriptional activator.

RBT1 deletion constructs were designed to determine the transactivating domain, and to define the domain which is essential for RPA32 interaction. The transactivation domain of RBT1 resides within 30 amino acids at the C-terminal. Truncation of RBT1 from the C-terminal end results in significant reduction of transactivation of the reporter genes.

A mammalian transactivation assay confirmed that a GAL4-RBT1 fusion protein indeed acts as a strong transcriptional activator. Furthermore, transcriptional activation, as assayed by a luciferase reporter gene, although high in all cancer cell lines examined, is at least 4 times higher in cell line MCF7. Transactivation studies were also performed using a mammalian system to verify that RBT1 acts as a transcriptional activator in its native cellular environment. RBT1, fused to a GAL4 DNA binding domain, strongly promotes transcription of

of B-gal activity with just 60 bp deleted from the 3', suggesting that the potential transcriptional activation domain of RBT1 lies at the carboxy terminal.

Similar constructs may be cloned into a vector for transfection into human cells, using an in-frame fusion to GAL4 DNA-binding domain and utilizing a second plasmid bearing a luciferase reporter gene under the control of several GAL4 binding sites. These experiments determine whether the transactivation found in the yeast system are physiologicallly relevant.

RBT1 may be overexpressed in various human cell lines to ascertain possible phenotypic effects. Experiments may include UV and chemical challenge.

Antibodies against RBT1 may be raised for subsequent protein localization experiments in human cells. This antibody may also be used for various co-immunoprecipitation experiments to show RPA-RBT1 binding.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth, and as follows in the scope of the appended claims.